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Use of Ectomycorrhizae (EM) in Restoration of Bottomland Hardwood (BLH) Wetland Forests

PURPOSE: This technical note characterizes EM colonization of common BLH tree seedlings experimentally planted in old agricultural fields and effects of inoculation with native wetland EM on growth and survival of the seedlings. Results of the experiment were used to develop recommendations for use of mycorrhizal inoculation in forested wetland restoration in the Southeast.

BACKGROUND: As presented in a companion technical note about EM of BLH wetland forests of the Southeast (Davis 1999), EM are a common symbiosis between fungi and tree roots (Figure 1). The fungi, which cannot photosynthesize, acquire carbon from the tree, and the tree benefits from absorption of extra nutrients and protection by the fungi. EM were shown to colonize a high percentage of root tips of common BLH wetland tree species in mature forests, even though the fungi are aerobic organisms (i.e., require oxygen).

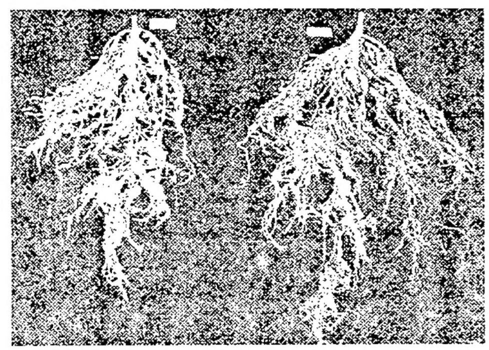

Figure 1. Ectomycorrhizae on root tips. Many EM are basidiomycetus fungi that form the familiar mushroom cap

EM fungi require tree hosts for growth and reproduction. Studies on severely disturbed lands found that mycorrhizal fungi were reduced or eliminated (Allen 1993). Removal of the host trees directly removes the nutrient source for the fungi. Indirectly, removal of the trees from the forest allows light to reach the forest floor and the soil to heat up and dry out. This creates inhospitable conditions for survival of mycorrhizal fungi in the soil. As a result, the fungi may not be present in denuded forests to colonize the planted tree seedling root systems.

The more extreme the environmental conditions in an area to be restored, the less likely the fungi will persist. Agricultural land in the Southeast that was converted from BLH wetland forests is exposed to extreme heat and moisture conditions. In addition, application of agricultural pesticides is likely to be detrimental to persistence of EM fungi hyphae in the soil. Many of the agricultural fields produced crops for more than 20 years prior to restoration, which is longer than the fungi spores are expected to persist. Loss of EM fungi from these restoration sites may be a limiting factor to survival and growth of planted BLH wetland tree seedlings.

Agricultural conversions are responsible for the loss of more than 25 percent of the 3,403,400 acres of forested wetland lost in the United States between mid-1970 and mid-1980 (Dahl and Johnson 1991). Several Federal agencies are very concerned about restoration of these agricultural lands, particularly in the Mississippi River Delta. To date, roughly 6,900 ha have been restored by the

Army Corps of Engineers,[1] 75,000 ha have been restored by the Natural Resource Conservation Service,[2] and 40,500 ha have been restored by the Fish and Wildlife Service.[3] The FWS has a goal to restore 405,000 ha of BLH wetland forest in the Mississippi River Delta for migratory waterfowl in the next 10-15 years with cooperation from other Federal agencies and private partners. Methods to improve the ecological and economic success of these restoration efforts are very important. Given the importance of the presence of the fungi to the health and growth of trees, this study investigated the role of the fungi in restoration of agricultural fields to BLH wetland forests.

METHODS: Water oak (*Quercus nigra*), nuttall oak (*Q. nuttallii*), and water hickory (*Carya aquatica*) seeds were germinated and grown as tublings (i.e., seedlings grown in tubes and planted with intact root systems) in sterile soil media. One half of the tublings were inoculated with one of two natural soils taken from two mature BLH wetland forests in Sharkey County, Mississippi. Noninoculated and inoculated tublings were planted in abandoned agricultural fields near the mature BLH wetland forest from which the soils were taken. The fields were very large, and to diminish effects of dispersal from the forest edge, tublings were planted > 0.8 km from the nearest forest edge.

Seedlings were measured for survival and height growth after one growing season. Roots were harvested and root tips examined to determine the percent colonized by EM. Selected representative colonized root tips were cultured. EM fungal colonies were characterized to species. These results were compared with EM fungal species cultured from roots of common BLH tree species (see Davis (1999)).

RESULTS: Inoculation of the tublings had essentially no effect on the morphology or colonization of the seedling roots when examined after one growing season (Table 1). A similar percentage of root tips were colonized on root systems with similar densities of root tips. The number of EM species cultured differed more between sites than between inoculated and noninoculated seedlings.

Important differences were noted, however, between the EM fungi characterizations from the mature BLH wetland forests and the seedlings from the agricultural fields (Table 1). Root tip colonization was nearly twice as high in the mature forests as in the agricultural fields. EM fungi species richness appeared to be comparable; the number of species being directly related to the number of roots cultured. The number of EM species isolated from one of the agricultural fields was low because of lower sampling intensity. There was little overlap in EM species isolated from any of the treatments: one species from one of the mature forests was also found in the nearby agricultural field, one EM species was found at both agricultural field sites, and four EM species were found on both inoculated and noninoculated seedlings.

A greater proportion of the EM fungi species grew "fast" in the agricultural fields than in the mature BLH wetland forest (Table 1). In addition, nearly twice as many cultured root tips from the agricultural fields produced EM cultures as did root tips from the mature BLH wetland forest, possibly

[1] Personal communication, 1997, Gary Young, Project Manager, U.S. Army Engineer District, Vicksburg, Vicksburg, MS.

[2] Personal communication, 1997, John DeFazio, Biologist, Natural Resources Conservation Service, New Albany, MS.

[3] Personal communication, 1997, Ron Haynes, Supervisory Biologist, U.S. Fish and Wildlife Service, Attauba, GA.

Table 1. Results of examinations of root tips from mature trees from BLH wetland forests and inoculated and noninoculated tublings planted in abandoned agricultural fields

	Mature Forest		Agricultural Field	
Parameter	**Inoculated**	**Noninoculated**	**Inoculated**	**Mean**
Number of root tips/1-cm root section	NA	11.0–11.7	10.9–11.5	11.4
Percent of root tips colonized per site	83–91	42–56	50–53	47–52
Mean	85	48	52	50
Number of EM species cultured per site	12–15	4–9	5–9	8–15
Mean	28	12	13	22
Percentage of successfully cultured root tips	21–30	NA	NA	50
Number of EM cultures with growth rate:				
Fast (> 70 mm dia/wk)	2–3	2–9	2–8	4–14
Mod. (25–70 mm dia/wk)	6–8	0	1–2	1–2
Slow (< 25 mm dia/wk)	6	0–2	0–1	0–3

indicating a more facultative relationship with the tree host. Slow-growing EM fungi species that are strongly dependent on their tree host are not easily cultured on an agar medium.

Measurements of growth and survival of the inoculated and noninoculated seedlings after one growing season showed no consistent effect. Height growth rates of the seedlings were not significantly different due to inoculation. Effects of inoculation on seedling survival appeared to depend on tree species, soil moisture, and site. For example, inoculated nuttall oak seedlings had significantly greater survival in dry areas of one agricultural field but the relationship did not hold on the second agricultural field. Survival of inoculated water oak seedlings was significantly reduced in dry areas of one site, but not in the other.

DISCUSSION: It is clear from the similar percentages of colonized root tips on inoculated and noninoculated seedlings that EM fungi are present in these abandoned agricultural fields. Lack of consistent improvement of survival or growth of the inoculated seedlings indicates that inoculation was not necessary in the agricultural fields to improve success of the planting. Results of these examinations, however, indicate an ecological succession of EM fungi that may have implications for ecological restoration of below-ground processes in the restored BLH wetland forests.

The pattern of species composition and growth rates among the EM fungi isolated from the agricultural fields and mature BLH wetland forests is very similar to early successional patterns above ground in abandoned fields. A wide variety of fast-growing weedy species colonize abandoned fields, but are soon replaced by a series of plant species that reduce light penetration to the soil, thereby reducing heat and moisture loss. Plant species that eventually develop into the mature plant community are capable of germinating and growing under these ameliorated conditions, whereas

the early successional plant species cannot survive. Late successional plant species frequently have slower growth rates and longer lives than early successional plant species.

The objective of planting tree seedlings in agricultural fields is to accelerate the successional process and restoration of the BLH wetland forests. Inoculation of the tublings with native soil, however, was not an effective means of accelerating the succession of EM fungi. The succession of EM fungi in restored agricultural fields will depend on whether the EM fungi species characteristic of mature BLH wetland forests remain in the agricultural soils or can disperse to the site. Further investigations will be required to more fully understand development of below-ground processes and their importance for ecological restoration of BLH wetland forests.

RECOMMENDATIONS: Results of these investigations indicate that inoculation with EM fungi is not required to improve the survival or growth of BLH wetland tree species if EM fungi are already present in the soil in sufficient amounts. The presence of EM fungi can be determined most expediently by growing noninoculated tree seedlings in soil from the restoration site, either in the field or greenhouse, and examining the root system.

Noninoculated tree seedlings must be grown from seed in a sterile soil medium. Seedlings should be allowed to grow in contact with the restoration site soil for a minimum of 8 weeks prior to examining the roots. Noninoculated tublings can be planted directly in the soil or a 20/80 mixture of the restoration site soil/sterile soil medium. The restoration site soil should be kept cool and not allowed to dry out. The roots must be gently washed to remove soil particles while keeping the root tips intact. Roots can be examined with the naked eye, or more easily, under a stereoscope. Colonized root tips appear to be enlarged with a fibrous coating that may be brown, black, white, or yellow (Figure 1). The percentage of colonized root tips gives a general idea of the number of EM colonizers available for the tree. Roots with fine root tips should be cut in 1-cm sections. Root tips and colonized root tips should be counted and averaged. Tree seedlings in this study had colonization rates greater than 40 percent. Colonization rates less than 20 percent may indicate the need for inoculation.

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POINTS OF CONTACT: For additional information, contact Dr. Judy Shearer (601-634-2516, *shearej@mail.wes.army.mil*). This technical note should be cited as follows:

> Davis, M. M., and Shearer, J. (1999). "Use of ectomycorrhizae (EM) in restoration of bottomland hardwood (BLH) wetland forests." *WRP Technical Notes Collection* (TN WRP VN-RS-4.3). U.S. Army Engineer Research and Development Center, Vicksburg, MS. *www.wes.army.mil/el/wrp*